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Plasma-Serum HSV-2 PCR Detection Kit

Product Insert

Product # 32500

Herpes Simplex Virus 2 (HSV-2) is a member of the herpes virus family, Herpesviridae. HSV-2 has a relatively large double-stranded DNA genome. HSVs are primarily transmitted by sexual intercourse, direct contact with lesions or perinatally. Most HSV positive cases are characterised by lesions on the skins and mucous membranes of the mouth and genitals. HSV infection can be either primary or a recurrence of a previous infection. More than 90% of the primary HSV infections are asymptomatic. Primary infection with HSV-1 can lead to gingivostomatitis, eczema herpeticum, keratoconjunctivitis and encephalitis. The primary symptoms of a secondary infection are skin lesions in the nose, mouth and genital regions. The infection is contagious, mainly during an epidemic.

Principle of the Test

Norgen's Plasma-Serum HSV-2 PCR Detection Kit constituents a ready-to-use system for the isolation and detection of HSV-2 using end-point PCR. The kit first allows for the isolation of total DNA, including viral DNA, from the Plasma-Serum samples using spin-column chromatography based on Norgen's proprietary resin. The viral DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for HSV-2 detection using the provided HSV-2 Master Mix. The HSV-2 Master Mix contains reagents and enzymes for the specific amplification of a 350 bp region of the HSV-2 viral genome. In addition, Norgen's Plasma-Serum HSV-2 PCR Detection Kit contains a second heterologous amplification system to identify possible PCR inhibition and/or inadequate isolation. The amplification and detection of either the *HSV-2 Isolation Control (IsoC)* or the *PCR control (PCRC)* does not reduce the detection limit of the analytical HSV-2 PCR. The kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Binding Solution I	6 mL
Proteinase K	1 vial
Pronase	1 vial
Binding Solution II	3 mL
Wash Solution I	4 mL
Wash Solution II	12 mL
Elution Buffer	3 mL
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
HSV-2 2x PCR Master Mix	0.35 mL
HSV-2 Isolation Control (IsoC)[a]	0.4 mL
HSV-2 Positive Control (PosC)[b]	0.1 mL
HSV-2 Negative Control (NegC)	1.25 mL
Norgen's DNA Marker	0.1 mL
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[a] *The positive control is a cloned HSV-2 product*
[b] *The isolation control is a cloned PCR product*

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- 96 – 100% ethanol
- 60°C incubator

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

Norgen's Plasma-Serum HSV-2 PCR Detection Kit contains ready-to-use Proteinase K and Pronase solutions, which are dissolved in a specially prepared storage buffer. The Proteinase K and the Pronase are stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K and Pronase, storage at 2–8°C is recommended.

The HSV-2 2x PCR Master Mix, the HSV-2 Isolation Control (IsoC), the HSV-2 Positive Control (PosC) and the HSV-2 Negative Control (NegC) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions while using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Plasma-Serum HSV-2 PCR Detection Kit, the HSV-2 2x PCR Master Mix, the HSV-2 Isolation Control (IsoC), the HSV-2 Negative Control (NegC) and the HSV-2 Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Plasma-serum HSV-2 PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Binding Solution I, Binding Solution II, Wash Solution I** and **Wash Solution II** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage

- Blood withdrawal causes injury of blood vessels (arteries, veins and capillaries).
- Only safe and sterile material should be used.
- For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed.
- Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand.
- Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 - 10 ml EDTA blood should be withdrawn.

Precaution: Samples of heparinised humans must not be used

2. Sample Storage

- Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 - 1,600 x g within six hours. The isolated plasma has to be transferred into sterile polypropylene tubes.
- The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.
- Virus encapsulated DNA is stable for days if stored at +4°C, for weeks if stored at -20°C and even for months and years when stored at -70°C.

3. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material
- We recommend sample transport with a courier. The blood samples should be shipped cooled (+2°C to +8°C) and the separated plasma deep frozen (-20°C).

4. Interfering substances

- Elevated levels of bilirubin (15 mg/dl) and lipids (800 mg/dl) and haemolytic samples do not influence the system.
- Heparin (10 IU/ml) affects the PCR. Samples, which have been collected in tubes containing heparin as an anticoagulant, should not to be used. Also, samples of heparinised patients must not be used.

B. Isolation of DNA from Plasma-Serum

Notes:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Always **vortex** both the **Proteinase K** and the **Pronase** before use.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of **Binding Solution II** and **Wash Solution I** by adding the proper volume of 96-100% ethanol indicated in Table 1 below (provided by the user) to the supplied bottle containing the concentrated **Binding Solution II and Wash Solution I**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Elevated levels of bilirubin (≥15 mg/dl) and lipids (≥800 mg/dl) and haemolytic samples do not influence the system

Table 1: Volume of Ethanol to be added to Binding Buffer II and Wash Buffer I

	Volume Provided	Ethanol (96-100%) Volume to Add	Final Volume
Binding Solution II	3 mL	7 mL	10 mL
Wash Solution I	4 mL	11 mL	15 mL

- An HSV-2 Isolation Control (*IsoC*) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the HSV-2 Isolation Control (*IsoC*) to the lysate during the isolation procedure.
    - The HSV-2 Isolation Control (*IsoC*) **must not** be added to the sample material directly
    - Do not freeze and thaw the HSV-2 Isolation Control (*IsoC*) more than 2 times.
    - The HSV-2 Isolation Control (*IsoC*) must be kept on ice at all times during the isolation procedure.

1. Add 200 µL of **Binding Solution I** for every 0.5 mL of Plasma-Serum sample. Mix well by inversion.
2. Transfer the 700 µL Plasma/Binding Solution I mixture into the provided spin column. Vortex for 15 seconds.
3. Centrifuge for 1 minute at 10,000 rpm and discard the flowthrough.
4. Add 30 µL of both **Proteinase K** and **Pronase** to column. **Vortex for 10 seconds**.
5. Incubate the mixture at **60ºC for 20 minutes**.
6. After the 20 minute incubation, add 260 µL **Binding Solution II**,
7. Add 15 µL ***HSV-2 Isolation Control (IsoC)*** to the lysate, mix well by vortexing.
8. Centrifuge for **1 minute at 10,000 rpm**. Do Not discard the flow-through.
9. Re-Load the flowthrough back to the column and centrifuge for **1 minute at 10,000 rpm**.
10. Apply 400 µL of **Wash Solution I** to the column and centrifuge for **1 minute at 14.000 rpm**. Discard the flowthrough and reassemble the spin column with its collection tube.
11. Apply 400 µL of **Wash Solution II** to the column and centrifuge for **1 minute at 14.000 rpm**. Discard the flow-through and reassemble the spin column with its collection tube.

12. Apply 400 µL of **96-100% Ethanol** to the column and centrifuge for **1 minute at 14.000 rpm**. Discard the flow-through and reassemble the spin column with its collection tube.

13. Spin the column for **1 minute at 14.000 rpm** in order to thoroughly dry the resin then incubate at 60^0C for 3 minutes. Discard the collection tube.

14. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 2,000 rpm,** followed by **1 minute at 14,000 rpm**.

C. HSV-2 PCR Assay Preparation

Notes:

- It is recommended that 10 µL of the DNA elution be used as the PCR sample input volume
- Sample volume can be varied between 2 µL – 10 µL of the DNA elution. PCR grade water should be added to make up the final volume of the PCR reaction to 20 µL.
- Using a lower volume from the sample than recommended may affect the sensitivity of the HSV-2 Limit of Detection.
- An HSV-2 Negative Control (*NegC*) and HSV-2 Positive Control (*PosC*) must be included during every run.
- The HSV-2 Negative Control (*NegC*) and HSV-2 Positive Control (*PosC*) provided are sufficient for eight PCR runs.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or quick vortexing), and centrifuged briefly.

1. Prepare PCR reactions as outlined in Table 2 below. For each sample to be run, pipette 10 µL of the eluted DNA and 10 µL of the Master Mix into a PCR tube. Each PCR reaction will have a final volume of 20 µL.
2. An HSV-2 Negative Control (*NegC*) and an HSV-2 Positive Control (*PosC*) must be included in every run. Pipette 10 µL of HSV-2 Negative Control (*NegC)* into a PCR tube and add 10 µL of Master Mix. Pipette 10 µL of HSV-2 Positive Control (*PosC)* into a PCR tube and add 10 µL of Master Mix.
3. Program the PCR machine according to the program shown in Table 3 below.
4. Run PCR.

Table 2: PCR Assay Preparation

Preparation of PCR assay	**Volume Per PCR Reaction**		
HSV-2 2X PCR Master Mix	10 µL	10 µL	10 µL
Sample (Eluted DNA)	10 µL		
HSV-2 Positive Control (PosC)	----------	10 µL	----------
HSV-2 Negative Control (NegC)	----------	----------	10 µL
Total Volume	20 µL	20 µL	20 µL

Table 3: HSV-2 PCR Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (40x)	**Step 1**	**94°C**	**10 sec**
	Step 2	**60°C**	**20 sec**
	Step 3	**72°C**	**30 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

D. HSV-2 PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE, 1.7% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The PCR products should be resolved on the 1X TAE, 1.7% Agarose gel at 150V for 30 minutes
- Figure 1 and Table 4 explain how to interpret the PCR assay results

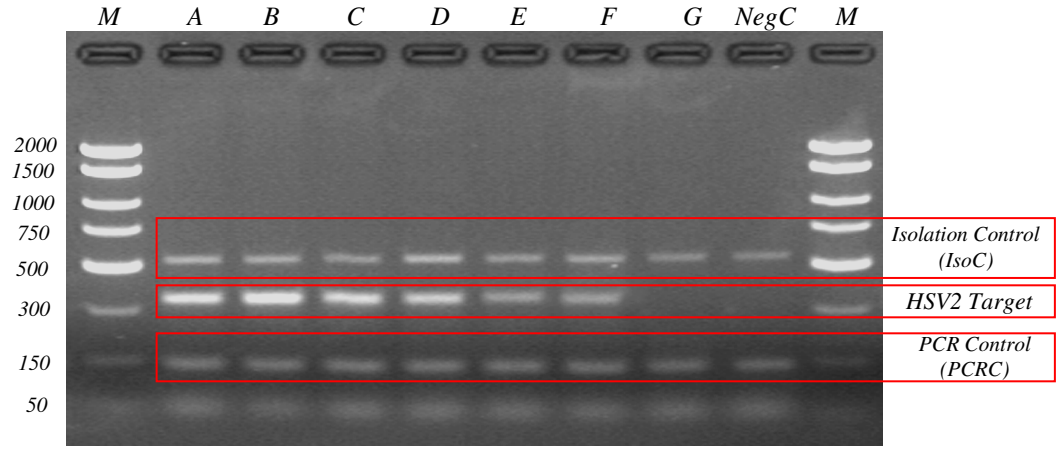

Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of HSV-2 at different concentrations (*HSV-2 target*). The size of the HSV-2 target amplicon corresponds to the 350bp band represented by the provided DNA Marker (M). The size of the HSV-2 Isolation Control (*HSV-2 IsoC*) corresponds to the 500bp band represented by the provided DNA Marker (M). The HSV-2 2X PCR Master Mix contains an HSV-2 PCR Control (*HSV-2 PCRC*). The HSV-2 PCRC Controls for PCR inhibition. The size of the HSV-2 PCRC corresponds to the 150bp band represented by the provided DNA Marker (*M*). Lanes A-G represents samples spiked with different HSV-2 concentrations isolated from 0.5mL Plasma (interpreted as positive results). The HSV-2 spiked in plasma samples is a cloned PCR product.

Table 4: Interpretation of PCR Assay Results

Input Type	HSV-2 IsoC Band (500 bp)	HSV-2 Target Band (350 bp)	HSV-2 PCRC Band (150 bp)	Interpretation
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample	X		X	Negative
Sample		X	X	Positive
Sample	X	X		Positive
Sample		X		Positive

** For results obtained that are not covered in Table 4 above, please refer to the Troubleshooting Section.

E. Specificity

- The specificity of Norgen's Plasma-Serum HSV-2 PCR Detection Kit is first and foremost ensured by the selection of the HSV-2-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses.

F. Linear Range

- The linear range (analytical measurement) of Norgen's Plasma-Serum HSV-2 PCR Detection Kit was determined by analyzing a dilution series of an HSV-2 quantitative standard ranging from 8.46×10^{9} VP/µl to 1×10^{-1} IU/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's Plasma-Serum HSV-2 PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen's Plasma-Serum HSV-2 PCR Detection Kit has been determined to cover concentrations from 0.2 VP/µl to at least 8×10^{6} VP/µl
- Under the conditions of Norgen's Plasma-Serum DNA Isolation procedure, Norgen's Plasma-Serum HSV-2 PCR detection Kit covers a linear range from 200VP/mL Plasma-Serum to at least 8×10^{9} VP/mL Plasma-Serum.

G. Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's Plasma-serum HSV-2 PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Negative Control and Positive Control are enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the HSV-2 PCR control nor the HSV-2 Isolation Control (IsoC) amplifies?

- If neither the HSV-2 PCR control nor the HSV-2 Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the HSV-2 PCR control showed amplification but neither the HSV-2 target nor the HSV-2 Isolation Control (IsoC) amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the HSV-2 Isolation Control (IsoC) was amplified in a sample?

- The sample tested can be considered as HSV-2 negative.

5. How should it be interpreted if only the HSV-2 target and the HSV-2 PCR control were amplified in a sample?

- The sample tested can be considered as HSV-2 positive.

6. How should it be interpreted if only the HSV-2 target was amplified in a sample?

- The sample tested can be considered positive. At high HSV-2 viral load, the HSV-2 amplicon will be predominant and the HSV-2 PCR control as well as the HSV-2 Isolation control may not amplify.

7. How should it be interpreted if only the HSV-2 PCR control and the HSV-2 Isolation Control (IsoC) showed amplification?

- The sample tested can be considered negative

8. Can I process a different Plasma-Serum volume?

- The reagents provided with the isolation kit are only sufficient to process 24 Plasma-serum samples of 0.5mL each.

9. What If I added more or less of the specified reagents' volume?

- Adding less volume may reduce your DNA yields. Adding more may not affect the DNA yields EXCEPT if more Elution Buffer was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.

10. What If my incubation temperature varied from the specified 60ºC?

- The incubation temperature can be in the range of 55°C - 65°C. At other temperatures the activity of both the Proteinase K and the Pronase will be reduced. This will result in a reduction in your DNA yields.

11. What If my incubation varied from the 20 minutes specified in the product manual?

- Less than 20 minutes will result in lower DNA yields. More than 20 minutes may not affect your DNA yields.

12. What If I forgot to do a dry spin after my second wash?

- Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with your down stream applications.

13. What If I forgot to add the HSV-2 Isolation control during the Isolation?

- The Isolation must be repeated.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma-Serum DNA Isolation Mini Kit (Slurry Format) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Related Products	Product #
Plasma-Serum Circulating RNA Isolation Kit	30000
Plasma-Serum Circulating Nucleic Acid Purification Kit	27800
Plasma-Serum Viral DNA Isolation Kit	29700
Plasma-Serum HSV-1 PCR Detection Kit	32700
Plasma-Serum HSV-1&2 PCR Detection Kit	31800

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